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Abstract

Background: It is imperative that all methods applied in skeletal age estimation and the criteria on which they are based have a strong evidential basis. The relationship between the persistence of epiphyseal scars and chronological age however has remained largely untested.

Aims: To assess the relationships between the level of persistence of the epiphyseal scar and chronological age, biological sex and side of the body in relation to the interpretation of epiphyseal scars in methods of skeletal age estimation.

Subjects and methods: A sample of radiographic images was obtained from the Tayside NHS Trust, Ninewells Hospital, Dundee, UK. This included images of four anatomical regions from living female and male individuals aged between 20 and 50 years.

Results: Some remnant of an epiphyseal scar was found in 78-99% of individuals examined in this study. The level of persistence of epiphyseal scars was also found to vary between anatomical regions.

Conclusion: The overall relationship between chronological age and the level of persistence or obliteration of the epiphyseal scar was found to be of insufficient strength to support a causative link. It is therefore necessary that caution is employed in their interpretation in relation to skeletal age estimation practices.

The epiphyseal scar: changing perceptions in relation to skeletal age estimation

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Introduction

The age of an individual is commonly interpreted as the elapsed time between their birth and the present. This is termed the chronological age. The relationship between the passage of time i.e. chronological age, and the development of the body exhibits a strong correlation; however some aspects of human development and maturation bear a closer relationship to the passage of time than others. Within the context of human identification, the most commonly examined areas of the body in relation to the estimation of chronological age are the dentition and the skeleton. Although it is generally accepted that the relationship between dental development and chronological age is closer than that observed between skeletal development and chronological age, there are significant limitations on the estimation of dental age in the process of human identification, particularly in relation to the susceptibility of the dentition to post-mortem loss (Đurić *et al.*, 2004, Hägg and Matsson, 1985, Lewis and Garn, 1960). For this reason, it is imperative that the information available in relation to all aspects of skeletal age estimation is tested and deemed accurate if it is to be used in a practical setting.

Skeletal age estimation is a fundamental component of the identification process associated with unidentified human remains. In recent years however, the use of age estimation has expanded and the frequency of application of skeletal age estimation in living individuals has increased. This is partially due to a rise in immigration of individuals of undocumented age or for whom the reported age is disputed (Ritz-Timme *et al.*, 2000, Schmeling *et al.*, 2007). The rise in the application of methods of skeletal age estimation to living individuals has led to a

concomitant rise in the number of publications related to this practice, particularly in relation to the estimation of age from the medial clavicle and the wrist. The rise in the use of medical imaging in relation to age estimation in living individuals has been accompanied by an increase in the use of radiographic and Computed Tomography (CT) imaging in deceased individuals. This has allowed practitioners to undertake skeletal assessments, including skeletal age estimation, through virtual means rather than through dissection or maceration (Brough *et al.*, 2012, Dedouit *et al.*, 2008, Dedouit *et al.*, 2007a, Dedouit *et al.*, 2007b, Telmon *et al.*, 2005).

A search of published English-language articles between 2010 and 2014 using Google Scholar and the search terms “Skeletal Age Estimation” + “wrist” yielded a total of 88 results, while the search term “Skeletal Age Estimation” + “medial clavicle” yielded 27 results. This is compared with a corresponding publication search between the years of 2000 and 2009 in which only 67 results were obtained from the former and 15 results for the latter of the search terms. To accommodate the variation in terminology, a second search of published English-language articles was conducted using the same year parameters and the search terms “skeletal age determination” + “wrist” and “skeletal age determination” + “clavicle”. This collection of search terms yielded 114 and 92 results for the period between 2000 and 2009 respectively while a total of 168 and 171 results were obtained respectively for the period between 2010 and 2014. Although there is a likelihood that some of these results will be duplicated by the use of the search terms “estimation” and “determination”, it is apparent that there has been a significant increase in the number of publications which relate to the

assessment of skeletal maturity in relation to chronological age in the years since 2010 compared to the previous decade.

As the number of extant publications increases, it is necessary to ensure that all available methods are supported by quantitative data and that sufficient information regarding the criteria on which they are based is available to enable practitioners to apply such methods in an appropriate manner. It is therefore imperative that the approaches to skeletal age estimation currently applied to living and deceased individuals are tested and the criteria on which they are based validated against relevant populations.

Radiographic methods of assessment, such as those relating to the hand and wrist (Andersen, 1971, Büken *et al.*, 2009, Bull *et al.*, 1999, Greulich and Pyle, 1950, 1959, Hackman and Black, 2013b, Schmeling *et al.*, 2006, Schmidt *et al.*, 2007, Schmidt *et al.*, 2008b, Tanner *et al.*, 2001, Tanner *et al.*, 1962, Tanner *et al.*, 1975, Thiemann and Nitz, 1991, Vignolo *et al.*, 1992), elbow (Brodeur, 1981, Diméglio *et al.*, 2005, Sauvegrain *et al.*, 1962), knee (Hackman and Black, 2013a, Pyle and Hoerr, 1969), and foot and ankle (Hackman *et al.*, 2013, Hoerr *et al.*, 1962) have undergone extensive testing on additional populations.

Comparatively few studies however have examined the validity of methods of age estimation based on specified maturity criteria and staging (Cameriere *et al.*, 2012, Davies *et al.*, 2013, O' Connor *et al.*, 2008). The paucity of research relating to the application of such methods raises questions pertaining to the validity of the assumptions on which the maturity criteria are based, including the assumption that those criteria developed in one anatomical region are directly applicable to others (Kellinghaus *et al.*, 2010, Schmeling *et al.*, 2004).

The epiphyseal scar is a feature commonly referred to in methods of skeletal age estimation that utilise maturity stages or criteria, particularly in methods pertaining to the hand and wrist (Baumann *et al.*, 2009, Schmidt *et al.*, 2008a). Previously termed “fusion lines”, epiphyseal scars have been observed in numerous anatomical regions including the long and short bones of the upper and lower limbs, and the medial aspect of the clavicle in both dry bone (Cope, 1920, Hall and Rosser, 1963, Klenerman, 1969, Klenerman and Marcuson, 1970, Workshop of European Anthropologists, 1980) and radiographic images (Baumann *et al.*, 2009, Davies *et al.*, 2014, Faisant *et al.*, 2014, O’ Connor *et al.*, 2008, Schmidt *et al.*, 2008a, Schulz *et al.*, 2008, Weiss *et al.*, 2012). The epiphyseal scar forms in the location of the former growth plate and appears as a band of bone of higher density than the cancellous structures on either side (Cope, 1920, Davies *et al.*, 2014, Faisant *et al.*, 2014). When viewed radiographically, this appears as a band of increased relative radio-opacity. As part of the internal structure of long bones, the epiphyseal scar is susceptible to modification from bone remodelling. Consequently, it has been assumed that the epiphyseal scar will, over time, become obliterated as a result of this process, and therefore that the presence of an epiphyseal scar is indicative of a younger individual (Workshop of European Anthropologists, 1980).

The obliteration of the epiphyseal scar is often cited as the final maturity indicator in methods of age estimation that utilise staging criteria (Baumann *et al.*, 2009, Schmidt *et al.*, 2008a, Whitaker *et al.*, 2002). Despite the use of this maturity criterion in multiple methods of age estimation from various regions of the skeleton, there is a lack of tangible evidence of a significant correlation between the obliteration of the epiphyseal scar and chronological age (Baumann

et al., 2009, Kellinghaus *et al.*, 2010, Schmidt *et al.*, 2008a). In contrast, several authors have, over the last century, reported the observation of epiphyseal scars in adults and the elderly in numerous anatomical regions including the proximal humerus, proximal and distal femur, proximal tibia and the first metatarsal (Cope, 1920, Hall and Rosser, 1963, Klenerman and Marcuson, 1970, Weiss *et al.*, 2012). Despite this, the observation of an epiphyseal scar has been interpreted as an indication of a younger chronological age since it is interpreted as evidence of recent epiphyseal fusion (Workshop of European Anthropologists, 1980). The lack of clarity surrounding the relationship between the persistence or obliteration of the epiphyseal scar and chronological age has potentially serious consequences for the process of skeletal age estimation in forensic cases in both living and deceased individuals. In cases involving deceased individuals, failure to accurately estimate the age of an unknown decedent may hinder the identification process resulting in not only complications for the investigating officers or agency, but also a protraction of the period of legal or emotional uncertainty for the next of kin (Ritz-Timme *et al.*, 2000). The risks associated with inaccurate estimations of age in living or deceased individuals are of sufficient concern to require an examination of the assumption that epiphyseal scars will become obliterated in the years following the completion of epiphyseal fusion. To this end, a study was undertaken to assess the degree of obliteration of epiphyseal scars observed in adult individuals. This study considered those areas of the skeleton commonly used in skeletal age estimation in living and deceased individuals including the distal radius, proximal humerus and proximal and distal tibia.

Materials and methods

A sample of 2452 radiographs from individuals aged between 20 and 50 years was obtained from the radiology department of Ninewells Hospital, Dundee, UK between December 2011 and March 2012. Although the ancestral origin of the individuals included in the sample was not known, data from the 2001 national census indicates that only 1.9% of the Tayside population is of non-European ancestry (The Scottish Government, 2010). Assuming an equal probability of attendance at hospital among all ancestral groups, there is no reason to consider that the ancestral distribution of the study sample differs from that of the region as a whole.

The sample used in this study included images from the proximal humerus, distal radius, proximal tibia and distal tibia that had been obtained for clinical assessment of injury between 2008 and 2011. To prevent the introduction of bias related to the duplication of individuals within the data set and to maintain a true cross-section of the clinical population, each individual was represented by a single radiograph within the study sample i.e. the sample represented 2452 individuals. Where possible, radiographs from 5 individuals were obtained for inclusion in each single year cohort however as this sample was obtained from existing clinical radiographs, the availability of suitable images was limited in certain cohorts. The distributions of the study sample according to anatomical region, chronological age and side of the body is presented in Table I.

All radiographs included in the study sample were clinically normal and showed no evidence of acute or previous trauma. This was determined through examination of the accompanying radiologist's reports. Any individuals for whom a history of chronic illness or injury affecting the areas of the bone under

consideration had been recorded were excluded from the study sample. This included those individuals with a history of delayed or precocious puberty; valgus or varus deformity of the knee or ankle; and hip dysplasia.

The date of birth (DoB) and biological sex of the individual from whom the radiograph had been obtained, the date on which image acquisition took place (DoI) and the side of the body from which the image was obtained were recorded for each radiograph included in the study sample. From this information, the chronological age of each individual, in years, was calculated using Microsoft Excel™. In accordance with data protection measures, each radiograph was anonymised and assigned a unique reference number (URN) according to the, biological sex of the individual, side of the body and the area of the body, followed by a sequential number e.g. MRDT1 would equate to image 1 of the data set obtained for the analysis of the male right distal tibia.

In preparation for the examination of the epiphyseal scar, each image was processed using Adobe Photoshop™ and was divided into six, equally spaced tracks numbered sequentially from the medial to lateral extremities of the bone. Examples of the track distributions for each of the proximal humerus, distal radius and proximal and distal tibiae are shown in Figures 1-4 respectively. Following the assignment of tracks, the degree of persistence of the epiphyseal scar in each track was assessed using a scoring system and the resulting scores were recorded in Microsoft Excel™. The criteria for the assignment of maturity scores are presented in Table II.

For each individual, the total persistence of the epiphyseal scar was calculated as the sum of the assigned maturity scores, termed the Total Persistence Score

(TPS). This score ranged between 0 in cases where the scar was found to be completely obliterated and 12 where the complete scar was retained. For each anatomical region, the percentage of individuals in whom some remnant of the epiphyseal scar was retained, termed the Total Persistence Rate (TPR) was calculated for females and males. Initial analysis was undertaken using a one-way Analysis of Variance (ANOVA) to assess the statistical significance of the variation in TPS between the left and right sides of the body. The relationship between TPS and chronological age, biological sex and side of the body was assessed through the application of General Linear Model (GLM) analyses. Subsequent GLM analyses were used to assess the relationship between TPS and anatomical region, chronological age, biological sex and side of the body from which the radiograph was obtained.

In addition to the calculation of TPS, regional persistence scores (RPS) were calculated for the medial, central, and lateral thirds of each bone. These values corresponded to the sum of the maturity scores assigned to tracks 1-2, 3-4 and 5-6 respectively. The mean RPS value was calculated for the medial, central and lateral regions of each bone in both sex cohorts for each anatomical region. The variation in the RPS values assigned to each region was assessed through the application of a series of one-way ANOVA. General linear model analyses were subsequently applied to each the data obtained from each anatomical area to determine the statistical relationship between chronological age, biological sex and side of the body on the regional persistence of the epiphyseal scar within the medial, central and lateral thirds of the bone. All statistical analyses were undertaken using IBM SPSS™ and Sigmaplot 12.0™ statistics software.

Intra-observer and inter-observer analyses

To assess the level of intra-observer variation in the assignment of maturity scores, a subsample of radiographs from 30 females and 30 males was re-assessed using the criteria outlined in Table III. The variation in the TPS values assigned on the first and second attempts was calculated in the female and male cohorts. The assigned maturity scores were considered to be in agreement if the assigned values were ± 2 scores. The percentage agreement between the assigned TPS scores was calculated. The statistical significance of the variation in the assigned TPS values was then calculated through the application of a series of one-way ANOVA. This process was repeated for each anatomical area examined in this study.

To assess the level of inter-observer variation in the assignment of maturity scores, the subsample of images examined during the intra-observer testing was assessed by three additional observers with varying levels of experience in skeletal age estimation and the interpretation of radiographic images. All three observers held a PhD in either human anatomy or forensic anthropology. Observer 1 had no background in skeletal age estimation or radiographic interpretation; observer 2 was a practicing forensic anthropologist who specialises in skeletal age estimation and observer 3 was a highly experienced forensic anthropologist. The percentage agreement (as defined in this study) was calculated for each pair of observers. This analysis was supported by the calculation of the statistical significance of the variation between the TPS values assigned by the observers. All statistical analyses undertaken to determine

intra-observer and inter-observer variation were conducted using IBM SPSS™ statistics software.

Results

Intra-observer analysis

The percentage intra-observer agreement between assessments was found to be $\geq 76.67\%$ in 7 out of 8 groups. The only cohort in which a percentage agreement of $< 76.67\%$ occurred was the female distal tibia. In this case, the percentage intra-observer agreement was 66.67%. The mean percentage agreement across all anatomical areas was approximately 78% in the female cohort while in the male cohort this increased to 80%.

Inter-observer analysis

Summaries of the percentage inter-observer agreement in each anatomical region in female and male cohorts are presented in Tables IV and V respectively. An initial analysis of the data from each anatomical indicated that that the greatest mean percentage inter-observer agreement exceeded 80% in both sex cohorts. Within the male sample, the greatest mean percentage inter-observer agreement was jointly observed in the proximal humerus and distal radius where 84.44% of assessments were within 2 scores. The highest mean percentage inter-observer agreement in the female sample was greater than that found in the male sample where 91.11% of assessments were within 2 scores of each other. Unlike the male sample, the greatest percentage agreement in the female sample was found in the proximal tibia. In both the female and male cohorts, inter-observer agreement exceeded 80% in a majority of anatomical

areas. The only exceptions to this were the distal radius (72.2%) and the proximal tibia (74%) in females and males respectively.

Analysis of the percentage agreement between pairs of observers was undertaken to establish the presence of any pattern in the consistency of assessments. Within the female sample, the percentage agreements observed between observers 1 and 2; and 2 and 3 were equal, with 85.83% of assessments being within 2 scores. The lowest percentage agreement occurred between observers 1 and 3 was 79.16%. Within the male sample, the greatest mean percentage agreement was found to occur jointly between observers 1 and 3 and 2 and 3 where 82.5% of assessments of TPS were within 2 scores. The lowest mean percentage agreement between a pair of observers occurred between observers 1 and 2 where 79.17% of assessments of TPS were within 2 scores.

A series of one-way ANOVA was conducted to establish the statistical significance of the variation in the TPS values assigned by each pair of observers. Within the female sample, only the variation in the TPS values assigned by observers 2 and 3 in the distal radius was found to exhibit a statistically significant degree of variation. Analysis of the data resulting from the assessment of the male cohort indicated that statistically significant degrees of variation between observers were restricted to the distal radius and proximal humerus. In both anatomical areas, the variation in the assignments of TPS by observers 2 and 3 was statistically significant. A series of one-way ANOVA was conducted to establish the statistical significance of the variation in the TPS values assigned by each pair of observers. Although some variation between assigned TPS values is present in some anatomical areas, the degree of inter-

observer variation in the female sample was not statistically significant in the majority of cases. The method may therefore be consistently applied to female individuals by multiple individuals. Within the male sample, the results of these analyses indicate that the variation between TPS values assigned by different observers is unlikely to be statistically significant in the lower limb; however there may be an increased risk of inter-observer disagreement in assessments of the upper limb.

Main analysis

Initial analysis was undertaken through the calculation of the TPR in each anatomical area. The results of this analysis, presented in Table VI, showed that the highest TPR was observed in the proximal tibia in both the female and male cohorts where values of 98.05% and 97.74% were achieved respectively. The lowest TPR was observed in the distal radius in both sex groups where values of 86.04% and 77.92% were found for females and males respectively. Further analysis of the raw data was undertaken to establish the percentage of individuals within 5 year cohorts were assigned a TPS value of ≥ 1 i.e. the percentage of individuals in whom some element of the epiphyseal scar was retained. The data pertaining to these analyses are summarised in Tables VII and VIII for females and males respectively. These analyses showed that between 82.5% and 96.7% of females aged between 45 and 50 years in all four regions retained some element of the epiphyseal scar. A similar level of persistence was found in the male sample where between 76.7% and 100% of males retained a portion of the epiphyseal scar. Results of the analysis of variation between left and right sides of the body indicated that while bilateral asymmetry was not

statistically significant in the upper limb, there was, significant variation between the left and right sides of the body in the proximal and distal tibia in both sex cohorts ($P \leq 0.001$), with the exception of the distal tibia in the female group.

The relationship between TPS and chronological age, biological sex and side of the body was examined further through the application of GLM analyses. A summary of the statistically significant results of these analyses is presented in Table IX. Analyses that did not render statistically significant results have been omitted from the table. The results of the GLM analyses indicate that biological sex exhibits a statistically significant relationship with TPS in the proximal ($P=0.045$) and distal tibia ($P=0.009$). This pattern was also observed in the relationship between TPS and chronological age where P values of 0.027 and 0.076 were found respectively. In addition to the bones of the lower limb, the relationship between chronological age and TPS in the proximal humerus was also found to be statistically significant ($P=0.025$). The relationship between side of the body and TPS was only statistically significant in the proximal tibia ($P=0.036$).

To assess the relationship between each of the factors examined in this study and the persistence of the epiphyseal scar, it was necessary to consider the value attained for the co-efficient of determination (R^2) of each interaction. Despite the occurrence of statistically significant interactions between TPS and biological sex; and TPS and chronological age in multiple anatomical regions, the maximum statistically significant R^2 achieved in any of these interactions was 0.076. This was observed in the relationship between TPS and chronological age in the distal

tibia. This finding suggests that chronological age, when considered as an independent variable, explains a maximum of 7.6% of the variation in the epiphyseal scar within the regions examined in this study.

It is not sufficient however to examine the effect of chronological age, biological sex and side of the body as independent factors since the effects of these influences may be inter-dependent. The results of this study indicated that the interaction between chronological age, biological sex and side of the body was statistically significant in the proximal humerus ($P < 0.001$), where this interaction explained 20.4% of the variation in the persistence of the epiphyseal scar in this anatomical region. Within the distal tibia, the highest statistically significant relationship was observed between biological sex and side of the body ($P = 0.001$). This interaction explained 27% of the variation in the persistence of the epiphyseal scar in the distal tibia. The strongest statistically significant relationship in the proximal tibia was observed between chronological age and biological sex ($P = 0.03$). Although this interaction was not the most statistically significant found in the proximal tibia, it explained the greatest variation in the persistence of the epiphyseal scar in this anatomical region ($R^2 = 0.101$). Within the distal radius, none of the factors examined in this study exhibited statistically significant relationships with the persistence of the epiphyseal scar when considered either independently or as co-varying influences.

The variation in the persistence of the epiphyseal scar between anatomical areas was quantified through the application of a further GLM analysis, the results of which indicated that the relationship between anatomical area and TPS was

statistically significant ($P < 0.001$). In addition, the variation in anatomical area was found to explain 15.2% of the variation in the persistence of the epiphyseal scar ($R^2 = 0.152$). Subsequent analysis of the complete data set indicated that the strength of the interaction between anatomical area and the persistence of the epiphyseal scar exceeded those found between TPS and chronological age ($R^2 = 0.021$), biological sex ($R^2 = 0.010$) or side of the body ($R^2 = 0.001$).

The potential effect of such factors on TPS was assessed through examination of the variation of TPS within three distinct regions of the epiphyseal scar in each anatomical area. The mean RPS values for each of the medial, central and lateral thirds in females and males were calculated for each anatomical area. These values are summarised in Table X. Within the bones of the upper limb, the highest mean regional persistence of the epiphyseal scar occurred in the central third of the bone in both females and males. The lowest RPS values for skeletal elements of the upper limb occurred in the medial third of each bone in both sex cohorts. With the exception of the lateral third in the proximal humerus ($P < 0.001$), the variation in RPS of the upper limb between females and males was not statistically significant. The pattern observed in the distribution of the highest and lowest mean RPS values in the upper limb was not replicated in the elements of the lower limb. Within the proximal tibia, the highest mean RPS occurred within the medial third of the bone in both sex cohorts. The lowest mean RPS within this anatomical area occurred in the lateral third of the bone in both females and males. The distribution of the epiphyseal scar in the distal tibia was less consistent than that observed in the proximal end of the bone. While the minimum RPS value occurred in the same region of the bone in both sex cohorts,

the location of the highest mean RPS value varied between females and males. Within the female sample, the highest mean RPS value occurred in the lateral third, while in the male cohort this occurred in the central third of the distal tibia.

Analysis of the variation in RPS between females and males was undertaken using one-way ANOVA in each of the anatomical regions considered by this study. The results of these analyses indicated that a statistically significant degree of variation in RPS between females and males was present in the lateral third of each bone examined in this study. This pattern indicates that the obliteration of the epiphyseal scar within the lateral third of each of the bones examined in this study may be influenced by localised factors which vary between females and males in both the upper and lower limbs. A statistically significant variation in RPS between females and males in the medial region was only observed in the proximal ($P < 0.001$) and distal ($P = 0.001$) tibia. This suggests that the degree of influence to which the medial regions of these bones are exposed varies between sexes. In a similar pattern to that observed in the medial regions no significant difference in RPS values assigned to the central third of the proximal humerus ($P = 0.071$) or distal radius ($P = 0.962$) was found. This was also observed in the distal tibia ($P = 0.464$). The absence of a statistically significant difference between the RPS values assigned to females and males in these anatomical regions indicates that the influences to which the epiphyseal scar in these regions is exposed do not vary significantly between the sex cohorts. In contrast, a statistically significant degree of variation was observed in the RPS values assigned to females and males in the proximal tibia ($P < 0.001$).

The variation between the medial, central and lateral regions of each bone in each sex was assessed through the application of a series of one-way ANOVA. With the exception of the variation between the central and lateral regions of the distal tibia ($P=0.081$) in the male sample, and the lateral and medial regions of the distal radius in both female ($P=0.201$) and male ($P=0.081$) cohorts, the variation in RPS between regions of each bone were statistically significant. In these cases the statistical significance of the variations ranged between 0.012 and <0.001 in the female sample and between 0.043 and <0.001 in the male sample.

A final GLM analysis was undertaken to establish the relationship between chronological age, biological sex, side of the body, area of the bone, region of the skeleton and the persistence of the epiphyseal scar (RPS). The results of these analyses indicated that the strongest explanatory model for the regional persistence of the epiphyseal scar included the factors of area of the skeleton, region of the bone and the biological sex of the individual ($P<0.001$; adjusted $R^2=0.196$). Despite the high degree of statistical significance exhibited by this model, the variation in RPS explained was less than 20%. The best explanatory model, inclusive of chronological age, which exhibited a statistically significant relationship with RPS, explained only 17.7% of variation in the regional persistence of the epiphyseal scar.

Discussion

It is imperative that the methods and standards employed to estimate the age of an individual are accurate, valid and based on sound scientific principles. Despite the use of the obliteration of the epiphyseal scar as the final maturity criterion in

methods of skeletal age estimation, there is a paucity of published evidence which supports the relationship between this feature and chronological age (Baumann *et al.*, 2009, Davies *et al.*, 2014, Faisant *et al.*, 2014, Schmidt *et al.*, 2008a, Whitaker *et al.*, 2002). Within the regions considered in this study, over 75% of individuals in all anatomical regions were found to retain some remnant of the epiphyseal scar. Total persistence rate was found to exceed 90% in 3 out of 4 of the anatomical areas considered in this study, with only that of the distal radius falling below 90%. As this study included individuals of up to 50 years of age, this initial finding indicates that complete obliteration of the epiphyseal scar is unlikely to occur in the majority of individuals. The percentage of individuals in whom some remnant of the epiphyseal scar was observed in this study exceeds that found by Weiss *et al.* (2012) in their study of the first metatarsal, in which, remnants of the epiphyseal scar were observed in 38% of individuals. The results presented in this study augment those published by Davies *et al.* (2014) and Faisant *et al.* (2014) in which the persistence of the epiphyseal scars of the proximal and distal tibia and the epiphyses of the knee joint respectively were reported to exceed 95%.

The highest overall TPR values for both sexes occurred within the proximal tibia while the lowest values were observed in the distal radius. As the obliteration of the epiphyseal scar can only occur as a result of skeletal remodelling, the variability in TPR values between the areas considered by this study and that of Weiss *et al.* (2012) indicate that remodelling of the cancellous structures, including the epiphyseal scar varies throughout the skeleton. This finding is supported within the literature where variation in the rate of skeletal remodelling within the skeleton has been acknowledged (Hsieh *et al.*, 2001). The

presence of variation in the persistence of epiphyseal scars throughout the skeleton may suggest that the remodelling of these features may be susceptible to influence from localised factors that vary between anatomical areas.

The distribution of TPR also suggests that the remodelling of epiphyseal scars increases in a proximal-distal direction. In both the upper and lower limbs, the findings of this study indicate that the more proximal elements exhibited higher TPR values than the more distal regions in each limb. This pattern may indicate that the rate of remodelling of epiphyseal scars is influenced by factors, the effects of which increase in the distal portions of the limbs, for example the application of mechanical loads. This hypothesis is consistent with the mechanostat theory that suggests that bone remodelling is influenced by the degree of mechanical loading to which the region is exposed (Frost, 1987, 2003). As the cumulative mechanical load to which the distal elements of each limb will exceed that to which the proximal limb sections are exposed, this may partially account for the variation in the degree of obliteration of the epiphyseal scars observed in this study.

In addition to the variation in TPR observed between anatomical regions, the statistical significance of the variation in TPS between males and females in each anatomical region was calculated. The results of these analyses indicated that this variation was only statistically significant in the proximal and distal tibia. The absence of statistically significant variation in TPS in the proximal humerus and distal radius suggests that the remodelling of the epiphyseal scar in these regions occurs at similar rates in both sexes. Conversely, the presence of statistically significant variation in TPS assigned to the bones of the lower limb suggests that the rate of remodelling of the epiphyseal scar within these skeletal

areas may vary between females and males. The variation in remodelling between sex cohorts may be partially attributable to the variation in normal calcium metabolism and circulating levels of systemic hormones.

The use of the epiphyseal scar in skeletal age estimation is reliant on the relationship that exists between the passage of time and the obliteration of the epiphyseal scar. The weak relationships observed between the persistence of the epiphyseal scar and chronological age, biological sex and side of the body in the anatomical areas considered in this study indicate that the majority of variation in TPS is attributable to factors other than those included in this study. The variation in the observed persistence of epiphyseal scars between anatomical areas may indicate that in addition to systemic influences e.g. calcium metabolism, more localised factors may affect the degree of persistence or obliteration of the epiphyseal scar. This study found that when the data set was examined in its entirety, the strength of the relationship between anatomical area and the degree of persistence of the epiphyseal scar exceeded those between TPS and any other factor examined. The interaction between anatomical area and TPS was highly significant, indicating that the degree of variation in the persistence of the epiphyseal scar was statistically significant. This supports the hypothesis that in addition to the systemic drivers of remodelling of the epiphyseal scar, localised factors may exert an influence on the degree of persistence of the epiphyseal scar. This is particularly evident in the distribution of the statistically significant interactions between biological sex and side of the body with TPS in the bones of the upper and lower limbs. The findings of this study suggest that the tibia, as a representative of the lower limb

skeleton, is more susceptible to influences attributable to these factors than the humerus or radius.

The regional persistence of the epiphyseal scar indicated that the greatest persistence of the epiphyseal scar within the upper limb occurred within the central thirds of the proximal humerus and distal radius. The variation in the persistence of the epiphyseal scar within the regions of the proximal humerus and distal radius was only statistically significant in the lateral third of each bone. This finding supports the potential role of localised factors on the remodelling of the epiphyseal scar. The absence of a statistically significant difference in the level of persistence between males and females in the medial and central thirds of the bones suggests that the remodelling of the epiphyseal scar in these regions may occur at a similar rate and that the localised factors to which these areas are exposed are similar in both sexes.

The greatest and lowest regional persistence of the epiphyseal scar in the proximal tibia occurred in the medial and lateral thirds respectively in both females and males. Although the regions in which the maximum and minimum mean persistence values occurred differed in the lower limb when compared with the upper limb, a similar pattern between females and males was observed. The pattern of remodelling observed in the proximal tibia, while different from that found in the upper limb, indicates that the influences to which the medial and lateral thirds of the proximal tibia are exposed may vary in both females and males. Further analysis however showed that the variation in TPS between females and males was statistically significant in all three areas of the proximal tibia. This may indicate that although the localisation of the influences to which

the proximal tibia is exposed is similar between the sexes, the effect that these factors have on the epiphyseal scar are significantly different in females and males.

Unlike the proximal humerus, distal radius or proximal tibia, no clear pattern in the mean regional persistence rate was observed in the distal tibia. Within the female sample, the highest mean RPS occurred in the lateral third of the bone while in the male sample this occurred in the central third. In both the female and male samples, the lowest RPS value occurred in the medial third of the bone. The low persistence rate observed in the medial third of the distal tibia may be attributable to the projection of the medial malleolus. As the placement of the track necessitated the use of the maximum width of the bone, the presence of a large medial malleolus may have removed the area of the epiphyseal scar from track 1. Consequently, the regional persistence rate for this area of the bone may, in some individuals, be represented by a single track rather than the sum of two tracks. Within the distal tibia, there was no statistically significant variation in RPS values assigned to the central third of the bone in females and males; however the variation between the sexes was significant in the lateral third. Subsequent analyses showed that the RPS values assigned to the lateral third in male individuals were not statistically different from those assigned to the central portion of the bone. This was not the case in the female sample where the variation between these regions was statistically significant. This suggests that in male individuals, the epiphyseal scar within the central and lateral thirds of the distal tibia are subject to similar degrees of influence.

The relationships between chronological age, biological sex, side of the body and RPS were assessed in the same manner as TPS. The results of these analyses indicated that the strongest model for the prediction of RPS included the factors of area of the skeleton, region of the bone and the biological sex of the individual. The addition of chronological age to this model negated the statistical significance of the previous model and resulted in a decrease in the co-efficient of determination and therefore the explanatory power of the model. These findings support those obtained from the analysis of TPS with respect to the effect of chronological age on the level of obliteration or persistence of the epiphyseal scar.

Conclusion

The observation of an epiphyseal scar on a radiographic image is a strong signifier of the completion of epiphyseal fusion and in this respect, has been incorporated into methods of skeletal age estimation in a number of anatomical areas, including those commonly examined in living individuals. There is however a degree of controversy relating to the length of time that an epiphyseal scar will remain visible on a radiographic image, or indeed on gross inspection of the bone itself.

While the inclusion of the observation of epiphyseal scars in methods of age estimation and the minimum age at which they become obliterated is not disputed, there is the potential for misinterpretation of such publications through the inclusion of a maximum age of persistence of epiphyseal scars. The consequences of this misinterpretation have the potential to be extremely serious in relation to the accuracy of age estimation for both deceased and living

individuals. It is therefore imperative that the potential persistence of epiphyseal scars in adult individuals is quantified.

It is apparent from the results of this study that epiphyseal scars may persist throughout the life of an adult individual. Although the maximum age of individuals included in this study was 50 years of age, the observation of epiphyseal scars in this age cohort indicates that these structures may remain visible well into the 6th decade of life. Analysis of the variation in the persistence of epiphyseal scars attributable to chronological age, biological sex and side of the body indicated that the strength of the interactions between these factors was insufficient to support a causative relationship. This is of particular importance in relation to the inclusion of maximum ages of persistence of epiphyseal scars in methods of skeletal age estimation. As the degree of persistence of epiphyseal scars appears to be largely independent of chronological age, it is recommended that where the observation of an epiphyseal scar is included in a method of age estimation, this is not accompanied by a maximum age. Similarly, due to the potential for misinterpretation, the inclusion of a mean chronological age for the persistence of epiphyseal scars is considered unwise.

Although epiphyseal scars were noted throughout the skeletal elements examined in this study, the degree of persistence was found to vary significantly between anatomical areas. This variation was also found to explain a greater degree of variation in the persistence of epiphyseal scars than chronological age, biological sex or side of the body, indicating that the persistence of epiphyseal scars may be partially affected by localised influences that differ between

anatomical areas. This may also suggest that methods of age estimation based on the remodelling of skeletal features may not be applicable to skeletal areas other than those on which they were developed.

In addition to elucidating the relationship between chronological age and the persistence of epiphyseal scars, the results of this study also indicate that the degree of persistence of epiphyseal scars may be under the influence of both systemic and localised factors. Further research is required to investigate the localised behaviour of epiphyseal scars and the potential influences to which these structures are exposed in adult individuals.

Declaration of Interest

The authors report no declarations of interest

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Table I Distribution of the study sample according to anatomical area, biological sex and side of the body

Region	Female Left	Female Right	Male Left	Male Right	Total
Prox. Humerus	155	155	154	155	619
Distal Radius	155	153	153	155	616
Prox. Tibia	155	153	154	155	617
Distal Tibia	152	150	149	149	600
Total	617	611	610	614	2452

Table II Scoring criteria for the epiphyseal scar

Score	Criteria
0	No epiphyseal scar observed within the track
1	A partial or fenestrated scar observed within the track
2	Epiphyseal scar completely traverses the track
X	No assessable bone present within the track

Table III Summary of intra-observer percentage agreement

Skeletal Area	Female	Male
Distal Radius	80.00	76.67
Proximal Humerus	86.67	80.00
Proximal Tibia	80.00	83.33
Distal Tibia	66.67	80.00
Mean	78.34	80.00

Table IV Summary of the percentage inter-observer agreement in the female sample, the overall mean percentage agreement between observer pairs and the mean inter-observer agreement in all skeletal areas

Skeletal Area	Observer 1 v Observer 2	Observer 1 v Observer 3	Observer 2 v Observer 3	Mean
Distal Radius	86.67	63.33	66.67*	72.22
Proximal Humerus	80.00	80.00	93.33	84.44
Proximal Tibia	86.67	86.67	100.00	91.11
Distal Tibia	90.00	86.67	83.33	86.67
Mean	85.34	79.17	85.83	83.61

*Statistically significant ($P \leq 0.05$)

Table V Summary of the percentage inter-observer agreement in the male sample, the overall mean percentage agreement between observer pairs and the mean inter-observer agreement for each skeletal area

Skeletal Area	Observer 1 v Observer 2	Observer 1 v Observer 3	Observer 2 v Observer 3	Mean
Distal Radius	93.33	83.33*	76.67*	84.44
Proximal Humerus	83.33*	83.33	86.67*	84.44
Distal Femur	53.33	73.33	80.00	68.89
Proximal Tibia	66.67	80.00	83.33	76.67
Distal Tibia	73.33	83.33	83.33	80.00
Mean	74.00	80.66	82.00	78.89

*Statistically significant ($P \leq 0.05$)

Table VI Summary of Total Persistence Rate according to biological sex and skeletal area

Skeletal Area	Female TPR	Male TPR
Distal Radius	86.04	77.92
Proximal Humerus	94.19	94.82
Proximal Tibia	98.05	97.74
Distal Tibia	92.72	92.95

Table VII A summary of the percentage of female individuals in whom a TPS ≥ 1 was observed according to 5-year cohorts

Age (years)	Prox. Humerus	Dist. Radius	Prox. Tibia	Dist. Tibia
20-24	98	84	100	96
25-29	94	88	100	95.9
30-34	88	95.8	96	100
35-39	96	76	100	92
40-44	100	90	96	91.8
45-50	90	83.3	96.7	82.5

Table VIII A summary of the percentage of male individuals in whom a TPS value of ≥ 1 was observed according to 5-year cohorts.

Age (years)	Prox. Humerus	Dist. Radius	Prox. Tibia	Dist. Tibia
20-24	100	80	98	91.7
25-29	94	72	100	94
30-34	96	80	94	90.1
35-39	96	80	100	91.5
40-44	92	79.2	93.8	90
45-50	91.5	76.7	100	98.3

Table IX Summary of the adjusted R² values of the relationships between Total Persistence Score and chronological age, biological sex and side of the body according to skeletal area

Skeletal Area	Age	Sex	Side
Distal Radius	0.011	0.004	-0.001
Proximal Humerus	0.025*	0.002	-0.001
Proximal Tibia	0.027*	0.045*	0.036*
Distal Tibia	0.076*	0.009*	0.002

*Statistically significant (P≤0.05)

Table X Summary of the mean Regional Persistence Scores in female and male individuals according to skeletal area

Skeletal Area	Medial		Central		Lateral	
	Female	Male	Female	Male	Female	Male
Distal Radius	1.08	1.09	1.41	1.26	1.17	0.95*
Proximal Humerus	0.75	0.76	1.74	1.84	1.16	0.89*
Proximal Tibia	2.37	2.02*	2.05	1.63*	1.64	1.41*
Distal Tibia	1.20	1.37*	1.97	2.04	2.73	1.86*

*Variation between females and males statistically significant ($P \leq 0.05$)

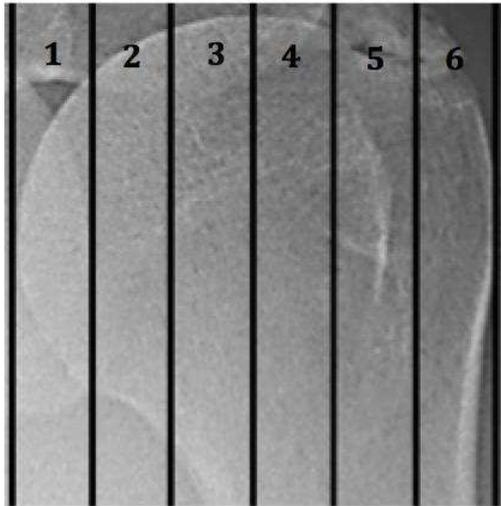


Fig 1 Track placement in the proximal humerus

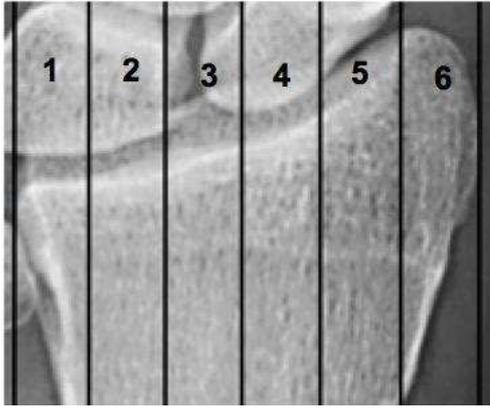


Fig 2 Track placement in the distal radius

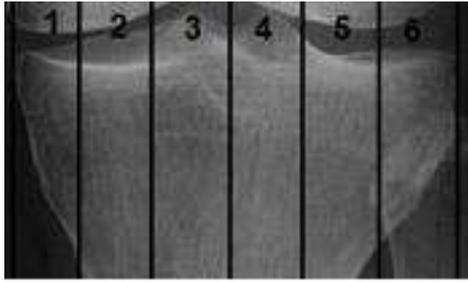


Fig 3 Track placement in the proximal tibia



Fig 4 Track placement in the distal tibia

Fig 1 Track placement in the proximal humerus

Fig 2 Track placement in the distal radius

Fig 3 Track placement in the proximal tibia

Fig 4 Track placement in the distal tibia